Gelatinases and their tissue inhibitors in a group of subjects with obstructive sleep apnea syndrome

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Abstract. Obstructive sleep apnea syndrome (OSAS) is associated with an elevated risk of cardiovascular events and stroke. Matrix metalloproteinases (MMPs) are endopeptidases involved in extracellular matrix degradation and then in the development and progression of cardiovascular diseases. Our aim was to evaluate plasma levels of gelatinases (MMP-2 and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2) in a group of subjects with OSAS. We enrolled 48 subjects (36 men and 12 women; mean age 49.7 ± 14.68 yrs) with OSAS diagnosed with a 1-night cardiorespiratory study and then we subdivided these subjects into two subgroups according to the apnea/hypopnea index (AHI): Low (L = 21 subjects with AHI <30) and High (H = 27 subjects with AHI >30). We measured plasma concentration of the gelatinases and their inhibitors using ELISA kits. We observed a significant increase in plasma concentration of MMP-9, MMP-2, TIMP-1 and TIMP-2 in the entire group of OSAS subjects and in the two subgroups, with higher levels in the H in comparison with the L subgroup. In the whole group of OSAS subjects we also noted a significant decrease in MMP-9/TIMP-1 ratio in comparison with normal controls. Only MMP-9 was significantly correlated with the severity of the disease, expressed as AHI, with the oxygen desaturation index and also with the mean oxygen saturation. MMPs pattern is altered in OSAS and significantly influenced by the severity of the disease; it probably contributes to the vascular remodeling that leads to the atherosclerotic disease and cardiovascular complications.

Keywords: MMP-2, MMP-9, TIMP-1, TIMP-2, obstructive sleep apnea

1. Introduction

The obstructive sleep apnea syndrome (OSAS) is a common sleep disorder characterized by repeated airflow obstructions during sleep with consequent episodes of apnea or hypopnea and intermittent arterial oxygen desaturation [8, 30]. The OSAS affects especially middle-aged and elderly subjects and its prevalence is increasing worldwide [27]. The gold standard methodology for diagnosis of OSA is the in-laboratory polysomnography, but the home testing with portable cardiorespiratory monitoring is an accredited alternative [8]. The frequency of respiratory events during sleep is expressed as apnea/hypopnea index (AHI), which defines OSAS severity [8]. The standard treatment for OSAS is the continuous positive airway pressure (CPAP) therapy, which ideally suppresses the apneic events [27].

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OSAS is independently associated with an increased risk of morbidity and mortality [28] considering that the most important complications are arterial hypertension, coronary artery disease, and cerebrovascular accidents [7, 21, 37, 39]. Atherosclerosis is common in OSAS, and several studies have demonstrated that the elevated mortality in OSAS is associated with the severity of the atherosclerosis [33]. In OSAS subjects the carotid intima-media thickness is significantly increased compared to controls [22] and a recent study has demonstrated that OSAS severity is correlated also with subclinical coronary atherosclerosis [38].

The mechanisms leading to the development and the progression of atherosclerotic plaques involve multiple factors, including oxidative stress, endothelial dysfunction, and inflammatory and immunologic factors. The continued hypoxia-reoxygenation episodes have a key role in the pathogenesis of the endothelial dysfunction in OSAS: the intermittent hypoxia may induce the production of reactive oxygen species (ROS) that contribute to the generation of adhesion molecules, leukocyte activation, and an enhanced systemic inflammation leading to endothelial damage [19].

Matrix metalloproteases (MMPs) are endopeptidases produced into the vascular wall, in particular by macrophages, able to degrade several extracellular matrix proteins [1, 12]. Gelatinases A and B (MMP-2 and -9) are responsible for IV type collagen, gelatin and laminin degradation, vasculature remodeling, angiogenesis, inflammation and then they are involved in the atherosclerotic process [10, 24]. Once they are secreted in the extracellular space, MMPs may be activated by several proteases, other MMPs, and ROS, in particular peroxynitrite [1, 15, 16]. Some authors have described an overexpression of MMP-9 in older atherosclerotic lesions [20], responsible for a large remodeling characterized by fibrosis, matrix degradation and angiogenesis, resulting in plaque instability and rupture [11, 20]. However, MMP-2 seems to be associated with a more stable plaque phenotype and rare haemorrhages [11]. MMPs activity is also regulated by the four tissue inhibitors of MMP (TIMPs): TIMP-1 inhibits especially MMP-9 while TIMP-2 inhibits especially MMP-2 [23]. An altered expression of MMPs and TIMPs has been observed in OSAS. Tazaki et al. observed a significant increase in MMP-9, but not in TIMP-1, serum levels in obese OSAS subjects compared to obese controls [32]. In addition, they found higher serum levels and activity of MMP-9 in subjects with moderate to severe OSAS than in subjects with mild disease [32]. MMP-9 concentration and activity were significantly correlated with AHI, BMI and inflammatory cytokines, such as IL-6 and TNF-α [32]. Similarly, Ye et al. [41] described elevated serum levels of MMP-9 in OSAS subjects in comparison with obese controls, correlated with OSAS severity and C-reactive protein levels. Differently, in children with OSAS no correlation between MMP-9 levels and OSAS severity has been observed [14]. Volná et al. [35] evaluated both the gelatinases in OSAS subjects and they observed a significant correlation only between MMP-9 and some polysomnographic parameters, such as oxygen desaturation index (ODI) and mean oxygen saturation. Chuang et al. [3] studied plasma levels of MMP-1, -2, -3, and -9 and of TIMP-1 and also plasma MMP-9 activity in OSAS subjects subdivided according to the severity of the disease; they found only elevated concentration and activity of MMP-9, especially after sleep. Tamaki et al. [31] observed in severe OSAS subjects an increased production of MMP-9 by monocytes immediately after the sleep, significantly decreased by long-term cPAP treatment. More recently, Vuralkan et al. [36] demonstrated a significant reduction of MMP-9 serum levels in OSAS subjects who underwent uvulopalatal flap surgery, but no correlation between post-operative MMP-9 and polysomnographic variables.

Previously we studied in subjects with OSAS the behaviour of nitric oxide metabolites (NOx) and erythrocyte deformability [2] and the relationship between OSAS severity and oxidative stress, examined as lipid peroxidation and protein oxidation [13]. In particular, we found a reduced red cell deformability, an altered NOx plasma level in subjects with severe OSAS, and an increase in lipid and protein oxidation, statistically correlated with the severity of OSAS [13]. Now our aim was to evaluate plasma concentration of MMP-9, MMP-2, and their tissue inhibitors (TIMP-1 and TIMP-2) in a group of subjects with OSAS.
2. Materials and methods

We consecutively recruited 48 subjects (36 men and 12 women; mean age 49.7 ± 14.68 yrs) with obstructive sleep apnea syndrome from those with suspected OSAS referred to our center. Clinical history and physical examination were performed in all subjects and Epworth Sleepiness Scale (ESS) was also given. OSAS was diagnosed after a 1-night cardiorespiratory sleep study: apneas were defined as the cessation of airflow for ≥10 seconds and hypopneas were defined as a transient reduction of breathing ≥50% with an oxygen desaturation of ≥3% or as a reduction of breathing ≥30% with an oxygen desaturation of ≥4% for ≥10 seconds. Obstructive apneas and hypopneas were distinguished from central events by the detection of respiratory efforts during the event. AHI was defined as the number of obstructive apneas and hypopneas per hour of sleep. Patients with an AHI ≥5 were considered as OSAS and then they were subdivided according to the AHI value in two subgroups: Low (L = 21 subjects with AHI <30) and High (H = 27 subjects with AHI ≥30). Means and S.D. of age, BMI, waist circumference, neck circumference, AHI, oxygen desaturation index (ODI), mean nocturnal SO₂ and mean heart rate (HR) are reported in Table 1 (Table 1); 23 of the OSAS subjects had arterial hypertension, 10 subjects had diabetes mellitus and 6 had cardiovascular disease (history of myocardial infarction or stroke). The control group consisted of 31 subjects (14 women and 17 men, mean age 41.3 ± 7.4 years) selected from the hospital staff; control subjects were free of medical diseases as assessed by clinical history, physical examination, electrocardiography, and routine hematological and urine analysis.

All the subjects gave their informed consent before entering the study and the study was approved by the Ethical Committee.

On fasting venous blood we evaluated plasma concentrations of gelatinases (MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) using respectively the Human MMP-2 ELISA and Human MMP-9 ELISA kit (Boster Biological Technology, LTD) and the Human TIMP-1 ELISA and Human TIMP-2 ELISA kit (Boster Biological Technology, LTD).

2.1. Statistical analysis

Data were expressed as means ± S.D. The statistical difference between the whole group of OSAS subjects and control subjects was evaluated using the Student’s t test for unpaired data. The statistical difference among control subjects, L subgroup and H subgroup of OSAS subjects was evaluated

| Table 1 | Mean ± S.D. of age, anthropometric characteristics and OSAS parameters in the whole group of OSAS patients and in the two subgroups with respectively mild and severe disease |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Age (years) | 49.7 ± 14.6 | 45.3 ± 14.4 | 52.8 ± 14.2 |
| BMI (kg/m²) | 35.4 ± 7.3 | 35.7 ± 8.5 | 35.1 ± 6.5 |
| Waist circumference (cm) | 118.8 ± 16.1 | 114.2 ± 14.5 | 122.5 ± 16.6 |
| Neck circumference (cm) | 44.4 ± 4.5 | 41.5 ± 3.2 | 46.6 ± 4.1*** |
| AHI | 38.5 ± 25.7 | 15.1 ± 8.1 | 56.6 ± 18.9*** |
| mSO₂ (%) | 91.1 ± 3.7 | 93.4 ± 2.7 | 89.5 ± 3.4*** |
| ODI | 39.3 ± 29.0 | 14.3 ± 9.4 | 55.4 ± 25.7*** |
| ESS | 11.1 ± 5.1 | 9.2 ± 3.7 | 12.4 ± 5.6* |

*p<0.05 ***p<0.001 vs mild OSAS (Student’s t test for unpaired data). BMI = Body Mass Index; AHI = Apnea-hypopnea index. mSO₂ = mean oxygen saturation. ODI = oxygen desaturation index. ESS = Epworth sleepiness scale.
employing the 1-way analysis of variance (ANOVA), integrated with the Bonferroni post-test. The correlations among MMP-2, MMP-9, TIMP-1, TIMP-2, demographic characteristics, and polysomnographic parameters were performed employing the linear regression test; the null hypothesis was rejected for \( p \) values <0.05.

3. Results

In the whole group of OSAS subjects we found a significant increase in plasma concentrations of gelatinases and their inhibitors in comparison with normal controls (Table 2). We noted also a significant decrease in MMP-9/TIMP-1 ratio compared to normal controls but no significant variation in MMP-2/TIMP-2 ratio (Table 2). Subdividing OSAS subjects in the two subgroups (L and H), we noted in both subgroups a significant increase of the examined parameters in comparison with normal controls (Table 3). We observed a significant variation of MMP-9 and TIMP-1 between the two subgroups (Bonferroni post-test) and in fact both these parameters were significantly higher in the H subgroup in comparison with the L subgroup of OSAS subjects (Table 3). In addition, we observed that the MMP-9/TIMP-1 ratio is reduced only in the L subgroup compared with normal controls (Table 3) while no statistical difference was found regarding the MMP-2/TIMP-2 ratio. About the demographic characteristics, in the entire group of OSAS subjects we observed a positive correlation between MMP-2 and age (\( r = 0.339, p < 0.05 \)), between TIMP-2 and age (\( r = 0.550, p < 0.0001 \)) and between MMP-9 and neck circumference (\( r = 0.403, p < 0.05 \)). Regarding the polysomnographic parameters...

Table 2
<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>All OSAS patients</th>
</tr>
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<tbody>
<tr>
<td>MMP-9 (ng/ml)</td>
<td>51.55 ± 8.14</td>
<td>99.12 ± 15.84***</td>
</tr>
<tr>
<td>TIMP-1 (ng/ml)</td>
<td>32.05 ± 4.80</td>
<td>67.98 ± 5.92***</td>
</tr>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>28.66 ± 4.15</td>
<td>35.78 ± 8.96***</td>
</tr>
<tr>
<td>TIMP-2 (ng/ml)</td>
<td>85.67 ± 9.40</td>
<td>105.8 ± 9.38***</td>
</tr>
<tr>
<td>MMP-2/TIMP-2</td>
<td>0.338 ± 0.059</td>
<td>0.337 ± 0.076</td>
</tr>
<tr>
<td>MMP-9/TIMP-1</td>
<td>1.652 ± 0.376</td>
<td>1.464 ± 0.234**</td>
</tr>
</tbody>
</table>

\( **p<0.01 \quad ***p<0.001 \) vs control subjects.

Table 3
<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Mild OSAS</th>
<th>Severe OSAS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9</td>
<td>51.55 ± 8.14</td>
<td>89.22 ± 11.07***</td>
<td>106.8 ± 14.8*** §</td>
<td>169.5b</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>32.05 ± 4.80</td>
<td>64.87 ± 5.53***</td>
<td>70.40 ± 5.09*** #</td>
<td>464.6b</td>
</tr>
<tr>
<td>MMP-2</td>
<td>28.66 ± 4.15</td>
<td>37.90 ± 10.44***</td>
<td>34.12 ± 7.39*</td>
<td>10.08b</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>85.67 ± 9.40</td>
<td>104.8 ± 8.35***</td>
<td>106.6 ± 10.19***</td>
<td>42.37b</td>
</tr>
<tr>
<td>MMP-2/TIMP-2</td>
<td>0.338 ± 0.059</td>
<td>0.361 ± 0.096</td>
<td>0.318 ± 0.050</td>
<td>2.351</td>
</tr>
<tr>
<td>MMP-9/TIMP-1</td>
<td>1.652 ± 0.376</td>
<td>1.387 ± 0.228**</td>
<td>1.523 ± 0.225</td>
<td>5.065a</td>
</tr>
</tbody>
</table>

\( ^a p<0.01 \quad ^b p<0.001 \) (ANOVA). \( ^* p<0.05 \quad ^** p<0.01 \quad ^*** p<0.001 \) vs control subjects (Bonferroni’s post-test). \( ^# p<0.01 \quad ^§ p<0.001 \) vs mild OSAS (Bonferroni’s post-test).
Fig. 1. Correlations between MMP-9 and polysomnographic parameters in the entire group of OSAS subjects.

(Fig. 1), we observed a positive correlation between MMP-9 and AHI value \( (r = 0.450, p < 0.01) \) and between MMP-9 and ODI \( (r = 0.36, p < 0.05) \) and a negative correlation between MMP-9 and mean oxygen saturation (mSO2) \( (r = -0.48, p < 0.001) \) in the whole group of OSAS subjects. No significant correlation among MMP-2, TIMP-1, TIMP-2 and polysomnographic parameters was observed.

4. Discussion

The obtained data showed an increase in plasma concentrations of gelatinases (MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in the entire group of OSAS subjects and in both the subgroups, although in the H subgroup the values of MMP-9 and TIMP-1 were significantly higher than in the L subgroup. While the MMP-2/TIMP-2 ratio did not discriminate the whole group and the two subgroups of OSAS subjects compared to normal controls, the MMP-9/TIMP-1 ratio was significantly reduced in the entire group and in the L subgroup of OSAS subjects. This behavior is probably imputable to the fact that the percentage increase in TIMP-1, observed both in the entire group and in the L subgroup, was surely higher if compared with the MMP-9 increase.

We retain that the behavior of the gelatinases is dependent especially on their over-production stimulated by the hypoxia-reoxygenation events and by several cytokines, such as IL-6 and TNF-\( \alpha \) [17, 18, 29, 36], while the trend of their tissue inhibitors might be imputable to their co-secretion or to a compensatory effect [6]. As well as the hypoxic stress induced by OSAS promotes the synthesis of inflammatory mediators [31, 35] involved in the development and the progression of the atherosclerotic disease, similarly the hypoxic stress seems to be able to modify the pattern of MMPs and TIMPs itself.

The behavior of MMP-2 and MMP-9 agrees partially with the results observed by other authors [3, 9, 32, 41] while our data regarding the tissue inhibitors are different from those of others [3, 32] who did not find any variation of these TIMPs between OSAS subjects and control groups. In agreement with Tazaki, Ye, Feng and Volna [9, 32, 35, 41], we observed in the whole group of OSAS subjects a positive correlation between MMP-9 and AHI, and between MMP-9 and ODI and also a negative correlation between MMP-9 and mSO2. In this study no significant correlation among MMP-2, TIMP-1, TIMP-2 and polysomnographic parameters was demonstrated.

Only in the whole group of OSAS subjects we also observed a positive correlation between MMP-9 and TBARS (thiobarbituric acid reactive substances) \( (r = 0.541, p < 0.001) \), between MMP-9 and carbonyl groups \( (r = 0.395, p < 0.005) \) and between MMP-9/TIMP-1 ratio and TBARS \( (r = 0.472, p < 0.0007) \); as it is known, TBARS reflects the lipid peroxidation while carbonyl groups express the protein oxidation. We did not find any statistical correlation between the other parameters of the oxidative stress (TAS, NOx) and the gelatinases or their inhibitors.

We know that OSAS is accompanied by several complications such as arterial hypertension, coronary disease and cerebrovascular events [7, 21, 37, 39], and it should be considered if the behavior of the
gelatinases might play a role in the development of these vascular complications. Up to now, the literature data show a controversial relationship between gelatinases and pulse wave velocity, that reflects arterial stiffness: in subjects with isolated systolic hypertension [40] and in subjects with chronic kidney disease, with or without diabetes mellitus [5], a correlation between gelatinases and arterial stiffness was observed, but not in healthy individuals [34] and in type 2 diabetic subjects [25]. In OSAS subjects it is not possible to exclude a role of the gelatinases in the development of arterial hypertension, although in this clinical condition the increase in blood pressure is imputable especially to the increase in sympatethic tone [5, 25, 34, 40].

Another consideration that regards the behaviour of gelatinases in OSAS subjects concerns their role about the angiogenesis. Gelatinases activity produces elevated levels of angiostatin through the proteolitic cleavage of plasminogen. As it is known, angiostatin inhibits endothelial cell proliferation and migration, induces apoptosis, reduces vascular endothelial growth factor expression and decreases eNOS activity [4]. It is possible that the influence played by the gelatinases on the angiogenesis might contribute to the increased risk of cardiovascular events, also considering that in this preliminar study MMP-9 and TIMP-1 clearly discriminate subjects with mild OSAS from subjects with severe OSAS, which have an higher cardiovascular morbidity and mortality. In OSAS subjects some authors [32] found a decrease in MMP-9 levels and activity after cPAP treatment, and others [31] noted even the decrease of MMP-9 production by monocytes after 3 months of cPAP therapy. Recently Vuralkan [36] demonstrated a decrease in MMP-9 levels in a group of OSAS subjects who underwent uvulopalatal flap surgery.

Regarding the MMPs pattern in OSAS, a point that deserves to be considered is if, in this clinical condition, MMPs may be retained a pharmacological target; we suppose that, in clinical practice, the several molecules that act on MMPs may give possible benefits in the overall treatment of this syndrome.

In summary, in OSAS we found an altered profile of the gelatinases and their inhibitors, and especially we observed that MMP-9 and TIMP-1, its principal inhibitor, are significantly higher in OSAS subjects with a severe degree of the disease in comparison with those of mild-moderate degree. In OSAS the evaluation of these parameters may be useful thus their behavior may contribute to explain in particular the vascular involvement that, in the course of time, accompanies this syndrome.

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References


E. Hopps et al. / MMPs and TIMPs in OSAS


